Chapter 11

Use of Animal Models in the Study of Colitis

Karoline S. Rezende, Melina R. Fernandes, Bernardo B. de Faria, Rita C. A. Guimarães and Karine C. Freitas

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75608

Abstract

Inflammatory bowel diseases (IBDs) relate to chronic inflammations in different parts of the gastrointestinal (GI) tract involving both ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis begins in the rectum and extends continuously up the colon. Notably, CD may affect any area of the GIT, from the mouth to the anus. Various conditions may influence the genesis of the disease, such as genetics, environment, intestinal microbiota and the presence of agents of enteric infections. Experimental models are therefore suitably used to investigate the various etiological factors; similarly colitis can be induced by genetic modification, cell transfer, spontaneous inflammation and chemical agents. The objective of this chapter is to present current concept on animal models of inflammatory bowel diseases. These models are crucial for the understanding of inflammatory bowel diseases, development of alternative treatments and more effective therapeutic agents thus contributing to the control of the disease.

Keywords: inflammatory bowel disease, animal models, ulcerative colitis

1. Introduction

The immune system has a mechanism of response to tissue injury and antigenic stimuli, whose main function is to protect the body. This response is called inflammation. It is characterized by the interaction of several types of cells and signaling molecules, to promote the repair of damaged tissue [1, 2]. Despite its protective function, the inflammatory response must not be prolonged, since exacerbated inflammation may cause physical loss of function in the organ [3].

The various manifestations characterized by chronic inflammation include inflammatory bowel diseases (IBDs), such as ulcerative colitis (UC) and Crohn's diseases (CD) [4, 5]. Their clinical presentations can be overlapping as well as distinct [6, 7]. UC is generally associated to superficial inflammation of the colon mucosa and CD comprises transmural damage that may occur in any segment of the gastrointestinal tract (GI) [8].

The IBDs are common among white people between the ages of 20 and 40 years old and the second peak of the disease occurs around 55 years of age. The distribution of this disease in males and females seems similar, though CD is more frequent in women [9].

The genesis of UC and CD involves multiple factors, such as genetic, environmental, intestinal microbiota and the presence of enteric infections. These factors may affect the immune system, by expanding and perpetuating the inflammatory process [10].

The treatment of IBD is considered unsatisfactory, because among other factors, cure may not be possible, only remission of the disease and/or treatment of symptoms. Several classes of drugs are currently used to treat IBD, such as aminosalicylates, corticosteroids and immunosuppressives [11]. The odds remain that these drugs have many side effects, are expensive and lose their efficacy after a certain period of medication [12].

Therefore, concise experimental studies are needed to unravel the etiology and pathophysiological mechanisms of IBD for development of alternative treatments and/or more effective therapeutic agents to treat patients with ulcerative colitis. Thus, the use of experimental animal models becomes essential to elucidate the mechanism(s) involved and accelerate the development of a specific and effective therapy.

2. Animal models for inflammatory bowel disease: colitis

The use of animal models is crucial for the investigation of the processes involved in the genesis of IBD. Although the development of the disease in animals does not present all the mechanisms observed in humans, animal models still are the most effective way to study the factors involved in the pathogenesis of BD. It would be difficult to conduct such a study in humans. Additionally, the animals are very useful for the assessment of new therapeutic approaches [13].

Over time, several experimental models of IBD were developed to conduct experimental studies, particularly mice and rats. The animal models of intestinal inflammation can be divided into four groups according to the type of induction namely: genetic modification, cell transfer, spontaneous inflammation and chemical agents [14–16].

The ideal experimental model is characterized by morphological disorders and intestinal inflammation, in addition to clinical symptoms, pathophysiology and development of the disease similar or equal to human condition IBD [17]. The chapter thus presents animal models developed for the study of and experimentation on colitis.

2.1. Genetic modification

The development and use of genetically modified animals have contributed to the investigation of various issues related to the IBD, and the most frequent are connected to the triggering of inflammation through the impairment of various functions such as epithelial barrier and detection of bacterial components, signaling of the innate immune response, immune regulation and signaling response to stress [18]. Studies involving the human genome have shown the association of more than 160 genes connected to IBD [19] (**Table 1**).

When the intestinal epithelial barrier is impaired, the intestinal mucosa has contact with bacteria from the lumen, which generates the inflammatory responses that will lead to the development of IBD. Studies have demonstrated the relationship between CD and certain genes involved in the intestinal epithelial barrier function. Animal models that express defects in this barrier are discussed and are essential in the investigation of IBD pathogenesis [18].

a. C57BL/6 mouse expressing a dominant negative N-cadherin (NCADΔ)

N-cadherin is a glycoprotein related to epithelial cell adhesion and is essential for normal development. Mice with a dominant-negative mutation of N-cadherin developed porous regions in the intestinal epithelium and intercellular adhesion disorder, developing transmural inflammation of the jejunum similar to CD at 3 months old. The organization of the small intestinal epithelium makes it possible to use chimeric mice, generated from normal blastocyts and genetically manipulated embryonic stem cells, to study cadherin function. Therefore, embryonic stem cells were transfected with a dominant negative N-cadherin mutant (NCAD*Δ*) under the control of promoters active in small intestinal epithelial cells and then introduced into C57BL/6 mouse blastocysts [20].

b. FVB mice

FVB mice originated from Swiss syngeneic strains subjected to processes that triggered intolerance or sensitivity to histamine. FVB mice were derived from the syngeneic Swiss strains, selected from crosses in which progenies received pertussis vaccination and challenged with histamine thus developing resistance or sensitivity to it. From this experimental system, two strains were selected: one resistant to the histamine factor (HSFR/N) and another sensitive (HSFS/N). Within the latter group, in the early 1970s, a group of eight syngeneic generations, HSFS/N, carrying Fv-1b alleles and susceptible to the Friend leukemia virus, lineage B, were selected. They are good breeders with large progenies and the eggs of the FVB/N lineage favor *in vitro* fertilization procedures since it has a huge pro-nucleus that facilitates DNA microinjection and the production of transgenic [21].

c. Muc2 knockout ($Muc2^{-/-}$) and Muc2 heterozygous ($Muc2^{+/-}$) mice

The surface of the intestinal mucosa has many components essential for its protection, such as MUC2 that has a lubricating function and also provides a protective barrier between the contents of the lumen and mucosal layers. Wild-type ($Muc2^{+}/^{+}$), $Muc2^{+}/^{-}$ and $Muc2^{-}/^{-}$ littermates are mostly used and scores recorded weekly until the age of 16 weeks to obtain a disease activity index (DAI). The previously mentioned $Muc2^{-/-}$ mice were backcrossed onto a 129SV

Mutation	Main results	References
Dominant-negative mutant N-cadherin	Transmural inflammation in the jejunum similar to that of patients with CD	[20]
Lack of Muc2	Development of IBD in the distal colon	[22]
Specific deletion of C1galt1 in epithelial cells	Inflammation in the distal colon	[23]
MDR1a gene deleted	Development of colitis	[24]
Absence of IRE1 α	Spontaneous colitis	[26]
Nuclear kappa B (NF-kB) deactivated in intestinal epithelial cells	Development of colitis with apoptosis of epithelial cells	[27, 28]
Specific deletion of type II receptor for TGB-β in CD4+-specific T cells and dendritic cells	Spontaneous inflammation in several organs and colitis	[29, 30]
Absence of SMAD4	Inflammation through the entire gastrointestinal tract	[31]
Absence of IL-2 and deficiency of JAK 3 (component of IL receptor)	Autoimmune disease with colitis and other disorders; spontaneous colitis	[32–34]
Absence of IL-10 and deletion of CRF2-4 (component of IL-10 receptor)	Spontaneous development of colitis; chronic colitis	[38, 39]
Absence of TCR α	Spontaneous triggering of colitis	[40]
Absence of STAT3 in macrophages and neutrophils	Spontaneous onset of colitis	[41]
Super expression of STAT4 and immunization with DNP-KLH/CFA	Development of transmural colitis	[43]
Absence of Gαi2	Triggering of colitis and colorectal cancer	[44–46]
Absence of enzyme A20	Inflammation in the intestine and in other organs	[47]
Absence of T-bet and T and B cells	Onset of colitis similar to UC	[48]
Altered levels of TNF-α	Development of inflammation of the ileum and in the distal colon	[49–51]
Super expression of IL-7	Spontaneous onset of colitis	[53]
Absence of Gpx1 and Gpx2	Triggering of colitis and ileitis	[54]
Specific deletion of XBP1 factor	Development of ileitis	[55]

Table 1. Genetic models of colitis.

(Charles River, Maastricht, The Netherlands) genetic background for nine generations followed by intercrosses to breed animals homozygous for the Muc2 disruption. Mice lacking this component may develop IBD in the distal colon at approximately 5 weeks of age [22].

d. *Intestinal epithelial cell-specific C1galt1$^{-/-}$ (IEC C1galt1$^{-/-}$) mice*

The enzyme β1, 3-galactosyltransferase 1 (C1galt1) is related to the synthesis of O-glycan in mucus-producing epithelial cells. The mucus transports a large amount of O-glycan, since

this substance forms 80% of the mucus molecular weight. Approximately 30% of the individuals with UC have disorder in O-glycan synthesis. To study the role of O-glycans in intestinal tissue, the model was generated by crossing mice with loxP sites flanking C1galt1 (C1galt1$^{f/f}$ mice) with an intestinal epithelium-specific Cre-expressing transgenic line (Villin-Cre mice), giving rise to animals lacking C1galt1 in the intestinal epithelium, referred to as C1galt1$^{-/-}$ (IEC C1galt1$^{-/-}$) mice specific for intestinal epithelial cells. All the animals with deletion of C1galt1 in intestinal epithelial cells developed distal colon inflammation at 12 weeks of age. Deletion of C1galt in epithelial cells was also induced in adult animals and, 10 days later, the development of colitis was also observed [23].

e. *MDR1a$^{-/-}$ mice*

The multiple drug resistance gene (MDR1) codes the multiple drug carrier (P-glycoprotein) in intestinal epithelial cells. Around 25% of FVB mice with the gene MDR1a "deactivated" developed colitis at 1 year of age [24]. In the Japanese population, the MDR1 gene was related to susceptibility to late onset of UC [25]. To obtain FVB.mdr1a−/− mice, FVB control mice and mdr1a$^{-/-}$ mice were bred and maintained in the SPF facility. Mdr 1a$^{-/-}$ mice had been backcrossed onto the FVB strain for at least seven generations for models obtained at Taconic Farms [24].

f. *Ire1 α $^{flox/flox}$-Villin-Cre mice*

Inositol requiring enzyme 1α (IRE1 α) acts as a major stress sensor. The Villin-Cre transgenic mice expressing Cre recombinase specifically in intestinal epithelium is currently available. Floxed mice (Ire1 α $^{flox/flox}$), in which the 121-nucleotide exon 2 of the Ern1 (i.e. Ire1 α) allele was flanked with two loxP recombination sites. Intestinal epithelia-specific Ire1 α knock-out mice (Ire1 α $^{flox/flox}$-Villin-Cre) were produced therefore, by intercrossing the Ire1 α $^{flox/flox}$ mice with Villin-Cre mice. Animals without IRE1 α develop spontaneous colitis and are characterized by the loss of goblet cells, which causes the intestinal epithelial barrier to become nonfunctional [26].

g. *NEMO^{IEC-KO} mice*

Animals with deactivation of nuclear factor-kappa B (NF-κβ) binding in intestinal epithelial cells, through conditional ablation of NEMO or both IKK1 (IKKa) and IKK2 (IKKb)—IKK subunits essential for NF-κβ activation, have an exacerbated response to luminal microbiota, which leads to the development of colitis with apoptosis of epithelial cells at the age of 6 weeks. To investigate the function of NF-κβ signaling in the gut epithelium *in vivo*, this was generated in mice lacking NEMO specifically in intestinal epithelial cells (NEMO^{IEC-KO} mice) by crossing mice carrying loxP-flanked (floxed, FL) NEMO (Ikbkg) alleles with Villin-Cre transgenics. These experiments were performed using mice backcrossed into the C57BL/6 genetic background for at least five generations. They were used as wild-type controls. The mice used were housed in individually ventilated cage systems, in either specific pathogen-free or conventional animal facilities [27, 28]. Therefore, regardless of the immune system, intestinal cells clearly provide protection against bacterial pathogens of the microbiota [18].

2.1.1. Transgenic animal models expressing colitis through modulation of immune regulatory agents

Mice with several genetic disorders associated with immune regulation were examined, revealing the development of colitis that was related to different substances [18]. These models with genetic modifications that affect immune regulation and trigger IBD are discussed below.

a. Type II receptor for TGF-β or SMAD4 absence

The transforming growth factor-beta 1 (TGF-β1) acts both in immune regulation and in the differentiation of regulatory T cells. Transgenic animals with dominant-negative mutant TGF-β type II receptor in specific CD4+ T cells showed spontaneous inflammation at around 3–4 months of age, and there was increased expression of Th1 and Th2 cytokines in several body organs such as colon, liver, stomach, duodenum, pancreas and kidney. These animals were created by using DNA microinjection into (C57BL/6xC3H) F1 fertilized oocytes, which results in six transgene-positive mice. All pups in the experimental cycle appeared normal at 2 weeks of age, but three died between 2 and 4 weeks of age. Three remaining transgene-positive mice appeared healthy and were bred; their progeny was further used to identify transgenic lines expressing a functional dominant-negative TGF-β receptor type II [29].

Animals with specific deletion of type II receptor for TGF-β in typical dendritic cells developed several inflammations, including colitis. Thus, B6.129S6-Tgfbr2^{tm1hlm} mice, carrying homozygous loxP site insertion flanking exon 2 of Tgfbr2 gene is currently available and obtainable from the National Cancer Institute (Frederick, MD) mouse repository (strain 01XN5) and other laboratories. CD11c-Cre transgenic mice (B6.Cg-Tg(Itgax-cre)1-1Reiz/J), OT-II transgenic mice (B6.Cg-Tg(TcraTcrb)425Cbn/J), Rag1$^{-/-}$ (B6.129S7-Rag1^{tm1Mom}/J) and wild-type C57BL/6 J are all obtainable by researchers. The mouse model with CD-specific T*gfbr2* deletion highlights the critical importance of TGF-β signaling in CDs in the maintenance of immune homeostasis and in the prevention of autoimmunity [30].

SMAD4 acts as a mediator in intracellular signaling of TGF-β. To inactivate specifically Smad4 in T cells, it was necessary to cross mice with a transgene encoding a Cre-recombinase driven by the Lck promoter (Smad4co/co; Lck-Cre) or the CD4 promoter (Smad4co/co; CD4-Cre) with the strain carrying the Smad4 conditional allele (Smad4co/co). Mice without SMAD4 in specific T cells and those without SMAD4 in CD4+ T cells had inflammation through the entire gastrointestinal tract at 3 months of age [31].

b. Mutations affecting the interleukin receptor (IL-2)

Interleukin-2 (IL-2) is related to activated-induced cell death and to the triggering of regulatory T cells function. Deficient IL-2 mice develop spontaneous autoimmune colitis, gastritis, hepatitis, pneumonia, pancreatitis, nephritis and hemolytic anemia [32]. Janus tyrosine kinase 3 (JAK 3) acts as a transducer of interleukin-2 receptor common gamma chain. Deficiency of JAK 3 led to the spontaneous development of colitis in mice [33]. The animal model that uses IL-2 is characterized by association of colitis with the autoimmune mechanism, which was demonstrated in the spontaneous onset of colitis without the presence of microorganisms [34].

Similarly, IL-10 is a regulatory cytokine with IBD susceptibility genes not only in adults but also in children [35–37]. IL-10 deficient mice develop spontaneous colitis [38]. Mice with deleted CRF2-4, a component of the IL-10 receptor, develop splenomegaly and disorders such as chronic colitis [39].

c. Mice with T cell-related mutations

The T cell receptor (TCR) is essential for the recognition of antigens by T cells. Approximately 60% of mice without TCR α, TCR component, developed Th2-mediated spontaneous colitis. The animals used in this study were produced by crossing TCR β × mutant mice with TCR mutant mice, and generated TCR β × gamma double mutant mice, which are deficient both in $\alpha\beta$ and γ cells. By mutating, the recombination-activating gene RAG-1 mice totally deficient in mature T and B lymphocytes were produced [40].

The transcription factor, signal transducer and activator of transcription 3 (STAT3) acts in the control of various innate immune responses such as differentiation of Th17 cells and epithelial renovation. Its gene is susceptible to UC and CD [35, 36]. Animals with intestinal epithelial cells deficient in STAT3 developed spontaneous colitis. In response to inflammatory stimuli, LysMcre/Stat$^{flox/-}$ mice develop enterocolitis even though they produce more IL-10 than normal animals in the same condition. To disrupt the Stat3 gene specifically in macrophages and neutrophils, two types of animals were crossed, mice in which the Stat3 gene is flanked by two loxP sites (Stat3$^{flox/flox}$) and the other mouse in which the Cre cDNA is inserted into the lysozyme M gene by a knock-in approach (LysMcre mice) [41].

STAT4 is a transcription signal transducer and activator factor that acts in the development of Th1 cells. Its gene was considered susceptible to UC in individuals with white skin [42]. Transgenic mice with super expression of STAT4 when immunized with 2,4- dinitrophenol-keyhole limpet hemocyanin/Complete Freund's Adjuvant (DNP-KLH/CFA) to activate the cytomegalovirus (CMV) developed transmural colitis. The murine STAT-4 cDNA was cloned downstream of the CMV promoter into the pcDNA3.1 expression vector (Invitrogen, San Diego, CA) yielding the pcDNA3.1S4 vector. A 3.6-kp *NruI*/*PvuII* fragment of pcDNA3.1S4 containing the STAT-4 expression cassette was microinjected into pronuclei of fertilized eggs of FVB/NHSD mice. Mice were maintained under specific pathogen-free conditions in isolated cages [43].

d. Gαi2$^{-/-}$ mice

Gαi2 signal transduction proteins participate in a wide signaling system. Mice that lack Gαi2 proteins showed dysfunctions related to T cells and developed colitis when they were 3–4 weeks old and colorectal cancer at approximately 3–4 months of age. Gαi2-deficient (Gαi2$^{-/-}$) mice on a 129SvEv X C57BL/6 background (backcrossed four or five generations into 129SvEv and then intercrossed) and on a pure 129SvEv background were used [44–46].

e. A20$^{-/-}$mice

A20 is an enzyme related to ubiquitin, which in turn is associated to NF-κβ1 activation. It acts in the prevention of tissue destruction and inflammation mediated by innate immune cells, in addition to regulating skin differentiation. To study the functions of A20 *in vivo*, A20-deficient

(A20$^{-/-}$) was generated by gene targeting. Rodents that lack this enzyme developed inflammation in various organs, including bowel, when they were 3–6 weeks olds [47].

f. T-bet$^{-/-}$ mice

T-bet is a transcription factor that regulates the differentiation of T cells into Th1 cells. To investigate whether the T-bet's protective function is linked to innate immunity, the T-bet$^{-/-}$ mouse developed on the RAG2$^{-/-}$ background was examined. The mice experimental model that lacked the T-bet factor showed no colitis. On the other hand, a new mouse model through which animals without T-bet were crossed with RAG2 mice lacking T and B cells showed the development of colitis similar to what occurs in UC at 4 weeks of age, therefore, T-bet expression protects against colitis and T-bet$^{-/-}$ × RAG2$^{-/-}$ mice develop spontaneous colitis [48].

g. TNF $^{\Delta ARE}$ mice

TNF-α is related to the onset of CD, because of the development of spontaneous inflammation in the ileum of mice with increased TNF-α activity [49]. Animals with intrinsic defect related to post-transcriptional regulation of TNF mRNA (TNF ΔARE rat model) developed inflammation, especially in the terminal ileum and subsequently in the proximal colon [50, 51]. To develop TNFΔARE mice, a 69bp deletion encompassing TNF ARE was conducted in embryonic stem cells in conjunction with the neomycin resistance marker (neo) flanked by loxP sequences. The excision of the neon gene and generation of TNFΔARE rat lines was due to the transient expression of the Cre recombinase in fertilized oocytes from the previously mentioned mice. The TNFΔARE homozygous mice developed early pathologies and also had a short shelf life. To reverse this problem, the effects of the TNFΔARE mutation on TNF biosynthesis were investigated in TNFΔARE/− hemizygous mice generated by the crossing of TNFΔARE/+ to TNFΔARE/− mice. The use of hemizygous TNFΔARE/− mice made it possible to investigate the regulatory role of the ARE element in a monoallelic system without the interference of a wild type TNF allele in the generation of TNF. So, a defective function of ARE may be etiopathogenic for the development of analogous human pathologies, in this case, Crohn's-like inflammatory bowel disease.

h. SRα/IL-7 mice

IL-7 has an important role in the homeostasis of T cells, and the gene linked to the IL-7 receptor was related to UC [35, 36]. To study the IL-7 role, it was critical to develop the model SRα/IL-7 transgenic mice using 2.1 kb SRα/lL-7 DNA fragment that was linearized by AatH and ApaU digestion. Microinjection into the pronuclei of fertilized eggs of C57BL/6 J mice was performed with ~10^3 copies of the SRα/lL-7 DNA fragment according to the standard procedure [52]. Rodents with super expression of IL-7 developed spontaneous colitis at about 4–12 weeks of age [53].

i. Double-KO mice deficient in both Gpx1 and Gpx2 gene expression

Activation of endoplasmic reticulum (ER) stress responses of intestinal epithelial cells to the metabolic by products of bacteria in the intestinal lumen is necessary for intestinal homeostasis and prevention of chronic inflammation [18]. The glutathione peroxidase (GPX) enzyme is

selenium-dependent and can reduce a broad range of hydroperoxides. The inflammatory process is associated with increased lipid peroxidation, and hydroperoxide appears to mediate IBD-related symptoms. To clarify the role of GPX in the intestinal epithelial cells, a homozygous double-KO mice with interruption in the expression of Gpx1 and Gpx2 genes was generated. These animals have signs and symptoms related to inflammatory bowel disease. The Ye-Shih Ho (Wayne State University, Detroit, MI) generated Gpx1-KO mice, as C57BL/6 J and 129Sv/J hybrids. Likewise, the generation of Gpx2-KO mice as B6 and 129S3 hybrids occurred at the City of Hope (COH; Duarte, CA) Transgenic Mouse Core. These mice were housed at the COH Animal Resources Center in ventilated cage racks. So, it was possible to verify that mice deficient in Gpx1 and GPX2 developed spontaneous colitis and ileitis GPX2 at about 3 weeks of age [54].

The transcription factor XBP1 is involved in the response to ER stress. XBP1 was related to CD and UC. To understand the function of this factor, Xbp1 exon 2 was almost completely disrupted in the intestinal tissue (small intestine and colon). Mice with specific deletion of this factor in intestinal epithelial cells developed spontaneous ileitis due to the lack of Paneth cells that secrete protective cells [55].

2.2. Adoptive transfer model of colitis

The referred methodology induces intestinal inflammation through selective transfer of certain types of immune cells in animals with impaired immune systems (RAG-1 knockout and RAG-2 knockout and SCID pigs). The study based on these models allows the investigation of immune factors, particularly the role of pathogenic T cells and T cell-mediated colitis on mucosal inflammation [18] (**Table 2**).

a. CD4+ CD45RB^{hi} model

The transfer of T CD4+ cells with high levels of protein CD45RB to mice deficient in T cells (RAG$^{-/-}$ or SCID) is the transfer methodology most commonly used to induce colitis, and it increases intestinal inflammation approximately 5–10 weeks after the transfer [56–58]. This method causes an inflammation similar to that of models that use chemical agents and is characterized by hyperplasia and inflammation in the colon, generally causing the death of the animal [59].

Models	Main results	References
CD4 + CD45RB^{hi}	Intestinal inflammation with Th1 responses to antigens of bacteria in the lumen	[56–61]
SheLAg-specific CD4+ T cells	Colitis caused by one single bacterial antigen	[62]
Hsp60-specific CD8+ T cells	Severe inflammation in the mucosa of the small intestine, regardless of the animal's microbiota	[63]

Table 2. Adoptive transfer models of intestinal inflammation.

This model of cell transfer triggers Th1 responses to bacteria in the lumen [60]. After cell transfer, naive T cells react to antigens of the luminal bacteria and expand rapidly in the lymph nodes and colon mucosa, becoming oligoclonal. The presence of pathogenic effector Th1 cells was possible due to the absence of regulatory T cells [18, 61].

b. SheLAg-specific CD4+ T cells

H. hepaticus Ag (SHelAg)-specific CD4+ Th1 clones transfer the disease to RAG1 knockout mice deficient in T cells and infected with a pathogenic bacterium (H. hepaticus) causing colitis between 7 and 8 weeks after the transfer, while the non-infected receptors did not develop colitis. It is inferred, therefore, that pathogenic T cells that respond to a single bacterial antigen may lead to the development of colitis [62].

c. Hsp60-specific CD8+ T cells

In general, heat shock proteins (HSP) are antigens considered potential targets to autoimmune disease. Then, the transfer of CD8+ T cells responsive to HSP60 for immunodeficient animals can trigger severe inflammation in the mucosa of the small intestine regardless of the animal's microbiota [63].

The severity of inflammation by adoptive transfer depends on the donor animal, as well as to the recipient [64, 65]. A study aimed to protocol standardization found that Balb/SCID mice develop a more severe and faster type of colitis C57BL/6 RAG-$1^{-/-}$ mice [66].

2.3. Models of spontaneous colitis

These models concern both the animals that develop spontaneous inflammation in the mucosa and those with inadequate mucosal immune response due to defect or genetic modification [56]. Spontaneous models are of great interest for the study of intestinal inflammation since, as in disease in humans, there is no external manipulation. The murine model C3H/HeJBir, for example, is characterized by chronic inflammation in the colon and in the cecal region [67] (**Table 3**).

a. C3H/HeJBir mouse model

Because of the occurrence of soft, light-colored feces and/or secondary perianal ulceration, and histological evidence of colitis, without pathogens, mice of the C3H/HeJ strain are likely to have a genetic predisposition to develop a form of IBD, with perianal ulceration, it has the pedigree to generate a "high-susceptibility" substrain, C3H/HeJBir, with a high incidence of spontaneous colitis [68].

Models	Main results	References
C3H/HeJBir	Chronic inflammation in the colon and cecal region similar to CD	[67, 69]
SAMP1/YitFc	Severe inflammation in the terminal ileum, similar to CD	[70, 71]

Table 3. Models of spontaneous colitis.

The wounds observed in this mouse model occur in the ileocecal region and the colon. Clinical manifestations begin at 3–4 weeks of age and last up to 10–12 weeks of age, approximately. The characteristics of the colitis developed by this methodology are similar to those of CD [17, 69].

b. SAMP1/YitFc model

Investigation of the mechanisms of CD can be based on the model that uses SAMP1/YitFc mice, where there is spontaneous and/or severe inflammation of the terminal segment of the ileum, which is the primary site of the wounds in CD, this disease model in animals also resembles human CD with regard to histologic features [15, 70, 71]. For the purposes of these experiments, a colony of SAMP1/Yit mice in a barrier facility at the University of Virginia was established from animals provided by Yakult Central Institute for Microbiological Research (Tokyo, Japan). The SAMP1/Yit (H-2^k) mouse strain was originally derived from AKR mice (original parents purchased from The Jackson Laboratories, Bar Harbor, Maine, USA) [70]. The findings from the adoptive transfer research have made it possible to infer that CD4+ T cells that generate a Th1-like cytokine profile, for example, IFN-γ and TNF, act as mediators of intestinal inflammation observed in SAMP1/Yit mice.

2.4. Chemically induced models of intestinal inflammation

Experimental models of colitis induced by chemical agents are used in the analysis of pathological mechanisms of IBD as well as the development of therapeutic agents. The referred method facilitates the induction of colitis. Some chemicals, such as dextran sodium sulfate (DSS) and trinitrobenzene sulfonic acid (TNBS) are the most commonly used substances. Models with such agents have been used for more than 20 years for the investigation and circumstances that affect the development of IBD [16] (**Table 4**).

Induction of inflammation by chemical agents can cause disorders in the barrier of the intestinal mucosa and/or cause hapten-induced hypersensitivity reactions [18]. Several factors are related to the magnitude of the onset of colitis in models induced by chemical substances. Although these models do not have the complexity of human IBD, they can contribute to the study of the processes involved in the control of the disease [72].

Models	Main results	References
DSS	Diarrhea with bleeding, ulcerations and infiltration of granulocytes. Some characteristics are similar to UC in humans	[73, 74]
TBNS	Transmural colitis that resembles CD	[80–82]
DNBS	Extensive tissue damage and acute inflammatory process similar characteristics to CD	[80]
Oxazolone	Inflammation of the distal colon with clinical similarities to UC	[74]

Table 4. Chemically induced models of intestinal inflammation.

a. DSS approach

DSS methodology has been used to induce colitis since 1985 because the disease induced is very similar to the human disease [73]. Its mechanism of action is related to direct epithelial toxicity, which impacts on the epithelial barrier of the intestinal mucosa [18]. In the referred methodology, the substance is diluted in water for animal drinking, for a period ranging from 5 to 7 days. The inflammation induced by DSS has characteristics similar to diarrhea with bleeding, ulcerations and granulocyte infiltration. Dysplasia often occurs during the chronic stage of the inflammation, which is very similar to ulcerative colitis in humans [16, 74].

The inflammatory immune response occurs immediately after disturbance of the intestinal epithelium, due to contact of the antigens in the lumen with the immune cells of the mucosa and submucosa areas [72].

Acute and chronic forms of the disease can be induced, according to the doses and treatment cycles. DSS was also used to induce IBD in transgenic and immunodeficient animals, and most of them are prone to intestinal inflammation. Also, the symptoms and the pathology are more severe with the association of this chemical agent [18]. DSS-induced colitis is frequently used in studies on innate immune processes involved in the onset of IBD and also in restoring the intestinal barrier integrity [72].

b. TBNS

In hapten-induced colitis, the TNBS substances dinitrobenzene sulfonic acid (DNBS) or oxazolone dissolved in ethanol can be administered rectally. The type of response triggered (Th1/Th2) will depend on the hapten selected, as well as on the susceptibility of the animal, and route of administration of the chemical agent. Ethanol is used to disrupt the barrier of the intestinal mucosa, while the agent (TNBS, DNBS or oxazolone) is associated with an autologous protein or microbiota byproducts, becoming immunogenic, and triggering the interaction of specific antigens and cells of the immune system [18, 75, 76]. The IBD models induced by hapten, similar to those that use DSS, can also be applied in transgenic and immunodeficient animals, as well as in those susceptible to immune-mediated colitis [77–80].

In the beginning of the experiment with TNBS-induced model, one single dose can be administered, resulting in acute inflammation, with Th1 response occurring in 2–3 days. Initial sensitization with TNBS can be done by rectal application or via skin, and another administration must be performed 6 days later for the development of delayed hypersensitivity response to TNBS-haptenized colonic proteins [81].

TNBS is administered rectally with ethanol, amidst haptenization of the proteins from the microbiota and the emergence of T CD4+ cells. The severity and the extent of the inflammation depend on genetic factors inherent in the animals and the presence or absence of bacteria that activate T cells [71]. T CD4 + cells are strongly related to Th1-mediated immune responses through IL-12 cytokines, culminating in transmural colitis similar to CD [80, 82].

The IBD model that uses DNBS is similar to the one induced by TNBS, because there is extensive tissue damage and acute inflammatory process. Also, as it occurs in CD, it depends on T CD4+ cells [80].

Oxazolone is generally administered after subcutaneous pre-sensitization (abdomen), which stimulates greater formation of Th2 cytokines and inflammation of the distal colon resembling ulcerative colitis [74].

Like TBNS, oxazolone is also administered rectally. Acute inflammation of the distal mucosa and submucosa generally involves ulcers and infiltrations of neutrophils, macrophages and lymphocytes. The type of response triggered depends on the dose of the substance administered. Overall, this model is useful for the investigation of specific characteristics of the inflammatory process in chronic IBD [72].

Type of induction	Characteristics	Limitations	Advantages	References
Genetic modification	Occurs by gene modification; overexpression or absence of a non-functional gene/protein of interest in all cell types or in a particular cell type, acting on the promotion of inflammation by impairment of epithelial barrier function and bacterial sensing, innate immune signaling, immune regulation, and stress response signaling	It analyzes the pathogenesis of IBD in general It tests the efficacy of new drugs in a lesser than other approached in a preclinical studies	Investigation of certain inflammatory mediators and cellular interactions present in intestinal inflammation Study and better understanding of the complexity of multiple genes and the different types of polymorphisms observed in patients	[14, 18, 19]
Chemical agents	It induces intestinal inflammation by acting on the disturbance of the mucosal barrier, epithelial and/or by triggering reactions of hypersensitivity by hapten	It is not sufficient to understand and characterize the inflammatory and immunological processes involved in intestinal inflammation and the pathogenesis of IBD	It encompasses methodologies that are considered easy to induce, and its simplicity allows it to be used in several experimental protocols	[14, 18]
Cell transfer	It induces intestinal inflammation in an immunodeficient host through the selective transfer of immune cell types	It uses a factor (adaptive immunity) that is not the only necessary requirement for the development of IBD	Investigation of specific route abnormalities and immune gut inflammation To study the role of regulatory T cells and mucosal immune regulation	[14, 18]
Spontaneous inflammation	The crossing of two types of rodents with different genetic contexts provided models that present spontaneous intestinal inflammation, without induced genetic alterations or interventions	Difficulty related to the acquisition of models (availability)	Because there is no intervention, it mimics the complex and multifactorial aspects that encompass the disease in humans	[14, 18, 66]

Table 5. Comparative presentation of the various methods of colitis induction.

Table 5 shows a comparative presentation of the various ways colitis disease is induced as discussed herein. Each of the several models discussed here have similarities with human IBD, and offer advantages for the study of certain aspects involved in the pathogenesis of the disease. Induction by chemical agents is often used widely by researchers mainly because it is easier, although it does not contribute to the elucidation of inflammatory and immunological processes involved in IBD [14, 18]. Genetic modification allows the study of specific aspects present in IBD; however, such form of induction does not allow to analyze the disease in a global way, and contributes less to the test of efficacy of new drugs [14, 18, 19]. Cellular transfer helps to study specific issues, contributing to the investigation of the role of regulatory T cells and mucosal immune regulation, but adaptive immunity alone does not act in the onset of IBD [14, 18]. The spine development of colitis reproduces the disease as in humans (since there is no intervention), allowing the investigation of complex and multifactor situations that are present in the disease, nevertheless, there is a low availability of models reported in the literature [14, 18, 66].

3. Final considerations

The animal models described here can contribute to the understanding of various mechanisms involved in IBD, as well as to the development of new drugs to improve the treatment. It would be extremely difficult to carry out these investigations and experimentation in humans. However, due to a good number of animal models available for this disease, care should be taken in the selection of animal/model choice. Investigators should take into consideration the purpose of their study, for example, the type of compound to be tested and/or pathophysiological mechanisms to be investigated.

Author details

Karoline S. Rezende[1]*, Melina R. Fernandes[1], Bernardo B. de Faria[2], Rita C. A. Guimarães[1] and Karine C. Freitas[1]

*Address all correspondence to: karolinesrezende@gmail.com

1 Post Graduation Program in Health and Development in the Middle West Region, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS, Brazil

2 Screenlab Laboratory, Campo Grande, MS, Brazil

References

[1] Ashley NT, Weil ZM, Nelson RJ. Inflammation: Mechanisms, costs, and natural variation. Annual Review of Ecology, Evolution, and Systematics. 2012;**43**:385-406

[2] Headland SE, Norling LV. The resolution of inflammation: Principles and challenges. Seminars in Immunology. 2015;**27**(3):149-160

[3] Dorward DA, Lucas CD, Rossi AG, Haslett C, Dhaliwal K. Imaging inflammation: Molecular strategies to visualize key components of the inflammatory cascade, from initiation to resolution. Pharmacology and Therapeutics. 2012;**135**(2):182-199

[4] Fiocchi C. Inflammatory bowel disease: Evolutionary concepts in biology, epidemiology, mechanisms and therapy. Current Opinion in Gastroenterology. 2013;**29**(4):347-349

[5] Mattos BRR, Garcia MPG, Nogueira JB, Paiatto LN, Albuquerque CG, Souza CL, et al. Inflammatory bowel disease: An overview of immune mechanisms and biological treatments. Mediators of Inflammation. 2015;**2015**:493012

[6] Gearry RB. IBD and environment: Are there differences between east and west. Digestive Diseases. 2016;**34**(1-2):84-89

[7] Zhai H, Liu A, Huang W, Liu X, Feng S, Wu J, et al. Increasing rate of inflammatory bowel disease: A 12-year retrospective study in NingXia, China. BMC Gastroenterology. 2016;**16**(1):1-7

[8] Judd TA, Day AS, Lemberg DA, Turner D, Leach ST. Update of fecal markers of inflammation in inflammatory bowel disease. Journal of Gastroenterology and Hepatology. 2011;**26**(10):1493-1499

[9] Arantes JAV, Santos CHM, Delfino BM, Silva BA, Souza RMM, Souza TMM, Cruz SBG, et al. Epidemiological profile and clinical characteristics of patients with intestinal inflammatory disease. Journal of Coloproctology. 2017:1-6

[10] Ferraz FB. Panorama Geral Sobre Doenças Inflamatórias Intestinais: Imunidade e Suscetibilidade da Doença de Crohn e Colite Ulcerativa. Journal of Health Science. 2016; **18**(2):139-143

[11] Dignass A, Lindsay JO, Sturm A, Windsor A, Colombel JF, Allez M, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 2: Current management. Journal of Crohn's and Colitis. 2012;**6**(10):991-1030

[12] Baumgart DC, Carding SR. Inflammatory bowel disease: Cause and immunobiology. Lancet. 2007;**369**(9573):1627-1640

[13] Hibi T, Ogata H, Sakuraba A. Animal models of inflammatory bowel disease. Journal of Gastroenterology. 2002;**37**(6):409-417

[14] Kolios G. Animal models of inflammatory bowel disease: How useful are they really? Current Opinion in Gastroenterology. 2016;**32**(4):251-257

[15] Jones-Hall YL, Grisham MB. Immunopathological characterization of selected mouse models of inflammatory bowel disease: Comparison to human disease. Pathophysiology. 2014;**21**(4):267-288

[16] Perše M, Cerar A. Dextran sodium sulphate colitis mouse model: Traps and tricks. Journal of Biomedicine and Biotechnology. 2012;**2012** 718617

[17] Jurjus AR, Khoury NN, Reimund JM. Animal models of inflammatory bowel disease. Journal of Pharmacological and Toxicological Methods. 2004;**50**(2):81-92

[18] Westbrook AM, Szakmary A, Schiestl RH. Mouse models of intestinal inflammation and cancer. Archives of Toxicology. 2016;**90**(9):2109-2130

[19] Mizoguchi A, Takeuchi T, Himuro H, Okada T, Mizoguchi E. Genetically engineered mouse models for studying inflammatory bowel disease. The Journal of Pathology. 2016;**238**(2):205-219

[20] Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. Science. 1995;**270**(5239):1203-1207

[21] USP, SPF-FVB [Internet]. Disponível em: http://www.biot.fm.usp.br/index.php?mpg=03.00.00&tip=CAMUNDONGO&id_ani=7&caract=sim [Acessed: 2017-09-11]

[22] Van der Sluis M, De Koning BA, De Bruijn AC, Velcich A, Meijerink JP, Van Goudoever JB, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology. 2006;**131**(1):117-129

[23] Fu J, Wei B, Wen T, Johansson ME, Liu X, Bradford E, et al. Loss of intestinal core 1-derived O-glycans causes spontaneous colitis in mice. The Journal of Clinical Investigation. 2011;**121**(4):1657-1666

[24] Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: Mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. The Journal of Immunology. 1998;**161**(10):5733-5744

[25] Arimura Y, Isshiki H, Onodera K, Nagaishi K, Yamashita K, Sonoda T, et al. Characteristics of Japanese inflammatory bowel disease susceptibility loci. Journal of Gastroenterology. 2014;**49**(8):1217-1230

[26] Zhang HS, Chen Y, Fan L, Xi QL, Wu GH, Li XX, et al. The endoplasmic reticulum stress sensor IRE1α in intestinal epithelial cells is essential for protecting against colitis. The Journal of Biological Chemistry. 2015;**290**(24):15327-15336

[27] Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature. 2007;**446**(7135):557-561

[28] Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, Du Y, et al. Epithelial-cell-intrinsic IKK-[beta] expression regulates intestinal immune homeostasis. Nature. 2007; **446**(7135):552-556

[29] Gorelik L, Flavell RA. Abrogation of TGFβ signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity. 2000;**12**(2):171-181

[30] Ramalingam R, Larmonier CB, Thurston RD, Midura-Kiela MT, Zheng SG, Ghishan FK, et al. Dendritic cell-specific disruption of TGF-β receptor II leads to altered regulatory T cell phenotype and spontaneous multiorgan autoimmunity. The Journal of Immunology. 2012;**189**(8):3878-3893

[31] Kim BG, Li C, Qiao W, Mamura M, Kasperczak B, Anver M, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. Nature. 2006;**441**(7096):1015-1019

[32] Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell. 1993;**75**(2):253-261

[33] Murata Y, Yamashita A, Saito T, Sugamura K, Hamuro J. The conversion of redox status of peritoneal macrophages during pathological progression of spontaneous inflammatory bowel disease in Janus family tyrosine kinase 3−/− and IL-2 receptor γ−/− mice. International Immunology. 2002;**14**(6):627-636

[34] Contractor NV, Bassiri H, Reya T, Park AY, Baumgart DC, Wasik MA, et al. Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice. The Journal of Immunology. 1998;**160**(1):385-394

[35] Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;**474**(7351):307-317

[36] Lees CW, Barrett JC, Parkes M. New IBD genetics: Common pathways with other diseases. Gut. 2011;**60**(12):1739-1753

[37] Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. The New England Journal of Medicine. 2009;**361**(21):2033-2045

[38] Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;**75**(2):263-274

[39] Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, et al. The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. The Journal of Experimental Medicine. 1998;**187**(4):571-578

[40] Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell. 1993;**75**(2):275-282

[41] Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Förster I, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity. 1999;**10**(1):39-49

[42] Liu QF, Li Y, Zhao QH, Wang ZY, Hu S, Yang CQ, et al. Association of STAT4 rs7574865 polymorphism with susceptibility to inflammatory bowel disease: A systematic review and meta-analysis. Clinics and Research in Hepatology and Gastroenterology. 2015; **39**(5):627-636

[43] Wirtz S, Finotto S, Kanzler S, Lohse AW, Blessing M, Lehr HA, et al. Cutting edge: Chronic intestinal inflammation in STAT-4 transgenic mice: Characterization of disease and adoptive transfer by TNF-plus IFN-γ-producing CD4+ T cells that respond to bacterial antigens. The Journal of Immunology. 1999;**162**(4):1884-1888

[44] Elgbratt K, Bjursten M, Willén R, Bland PW, Hörnquist EH. Aberrant T-cell ontogeny and defective thymocyte and colonic T-cell chemotactic migration in colitis-prone Gαi2-deficient mice. Immunology. 2007;**122**(2):199-209

[45] Huang TT, Zong Y, Dalwadi H, Chung C, Miceli MC, Spicher K, et al. TCR-mediated hyper-responsiveness of autoimmune Gαi2−/−mice is an intrinsic naïve CD4+ T cell disorder selective for the Gαi2 subunit. International Immunology. 2003;**15**(11):1359-1367

[46] Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, et al. Ulcerative colitis and adenocarcinoma of the colon in Gαi2-deficient mice. Nature Genetics. 1995; **10**(2):143-150

[47] Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-κB and cell death responses in A20-deficient mice. Science. 2000; **289**(5488):2350-2354

[48] Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. 2007;**131**(1):33-45

[49] Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: Implications for joint and gut-associated immunopathologies. Immunity. 1999;**10**(3):387-398

[50] Apostolaki M, Manoloukos M, Roulis M, Wurbel MA, Müller W, Papadakis KA, et al. Role of β7 integrin and the chemokine/chemokine receptor pair CCL25/CCR9 in modeled TNF-dependent Crohn's disease. Gastroenterology. 2008;**134**(7):2025-2035

[51] Kontoyiannis D, Boulougouris G, Manoloukos M, Armaka M, Apostolaki M, Pizarro T, et al. Genetic dissection of the cellular pathways and signaling mechanisms in modeled-tumor necrosis factor–induced Crohn's-like inflammatory bowel disease. The Journal of Experimental Medicine. 2002;**196**(12):1563-1574

[52] Uehira M, Matsuda H, Hikita I, Sakata T, Fujiwara H, Nishimoto H. The development of dermatitis infiltrated by γδ T cells in IL-7 transgenic mice. International Immunology. 1993;**5**(12):1619-1627

[53] Watanabe M, Ueno Y, Yajima T, Okamoto S, Hayashi T, Yamazaki M, et al. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. The Journal of Experimental Medicine. 1998;**187**(3):389-402

[54] Esworthy RS, Aranda R, Martín MG, Doroshow JH, Binder SW, Chu FF. Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2001;**281**(3):G848-G855

[55] Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell. 2008;**134**(5):743-756

[56] Borm ME, Bouma G. Animal models of inflammatory bowel disease. Drug Discovery Today: Disease Models. 2004;**1**(4):437-443

[57] Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD. CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. The Journal of Experimental Medicine. 1993;**178**(1):237-244

[58] Powrie F, Leach MW, Mauze S, Caddie LB, Coffman RL. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. International Immunology. 1993;**5**(11):1461-1471

[59] Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. Gastroenterology. 1995;**109**(4):1344-1367

[60] Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. The Journal of Immunology. 1997;**158**(7):3464-3473

[61] Wirtz S, Neurath MF. Animal models of intestinal inflammation: New insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease. International Journal of Colorectal Disease. 2000;**15**(3):144-160

[62] Kullberg M, Andersen J, Gorelick P, Caspar P, Suerbaum S, Fox J, et al. Induction of colitis by a CD4+ T cell clone specific for a bacterial epitope. Proceedings of the National Academy of Sciences. 2003;**100**(26):15830-15835

[63] Steinhoff U, Brinkmann V, Klemm U, Aichele P, Seiler P, Brandt U, et al. Autoimmune intestinal pathology induced by hsp60-specific CD8 T cells. Immunity. 1999;**11**(3):349-358

[64] Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Thl responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity. 1994;**1**(7):553-562

[65] Powrie F. T cells in inflammatory bowel disease: Protective and pathogenic roles. Immunity. 1995;**3**(2):171-174

[66] Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, et al. T cell transfer model of chronic colitis: Concepts, considerations, and tricks of the trade. American Journal of Physiology - Gastrointestinal and Liver Physiology. 1999; **296**(2):G135-G146

[67] Pizarro TT, Arseneau KO, Bamias G, Cominelli F. Mouse models for the study of Crohn's disease. Trends in Molecular Medicine. 2003;**9**(5):218-222

[68] Sundberg JP, Elson CO, Bedigian H, Birkenmeier EH. Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. Gastroenterology. 1994;**107**(6):1726-1735

[69] Cong Y, Brandwein SL, McCabe RP, Lazenby A, Birkenmeier EH, Sundberg JP, et al. CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: Increased T helper cell type 1 response and ability to transfer disease. The Journal of Experimental Medicine. 1998;**187**(6):855-864

[70] Kosiewicz MM, Nast CC, Krishnan A, Rivera-Nieves J, Moskaluk CA, Matsumoto S, et al. Th1-type responses mediate spontaneous ileitis in a novel murine model of Crohn's disease. Journal of Clinical Investigation. 2001;**107**(6):695-702

[71] Matsumoto S, Okabe Y, Setoyama H, Takayama K, Ohtsuka J, Funahashi H. Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. Gut. 1998;**43**(1):71-78

[72] Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, et al. Chemically induced mouse models of acute and chronic intestinal inflammation. Nature Protocols. 2017;**12**(7):1295-1309

[73] Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990;**98**(3):694-702

[74] Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. The Korean Journal of Physiology and Pharmacology. 2014;**18**(4):279-288

[75] Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. Nature Protocols. 2007;**2**(3):541-546

[76] Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology. 1989;**96**(3):795-803

[77] Iijima H, Neurath MF, Nagaishi T, Glickman JN, Nieuwenhuis EE, Nakajima A, et al. Specific regulation of T helper cell 1-mediated murine colitis by CEACAM1. The Journal of Experimental Medicine. 2004;**199**(4):471-482

[78] Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W. Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity. 2002;**17**(5):629-638

[79] Camoglio L, Velde AAT, Boer AD, Kate FJT, Kopf M, Deventer SJV. Hapten-induced colitis associated with maintained Th1 and inflammatory responses in IFN-g receptor-deficient mice. European Journal of Immunology. 2000;**30**(5):1486-1495

[80] Qiu BS, Vallance BA, Blennerhassett PA, Collins SM. (1999). The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. Nature Medicine. 1999;**5**(10):1178-1182

[81] Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(21):7671-7676

[82] Neurath MF, Fuss I, Kelsall B, Büschenfelde KH, Strober W. Effects of IL-12 and antibodies to IL-12 on established granulomatous colitis in mice. Annals of the New York Academy of Sciences. 1996;**795**(1):368-370